



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of : **Confirmation No. 8311**
Hirotaka KAKITA et al. : **Attorney Docket No. 2006_1644A**
Serial No. 10/594,899 : **Group Art Unit 1651**
Filed September 29, 2006 : **Examiner Sheridan R. MacAuley**
IMMATURE UNIALGAL CULTURE
STRAIN : **Mail Stop: AF**

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, **Mr. Hirotaka Kakita**, the undersigned, a citizen of Japan, residing at **Takamatsu-shi, Kagawa, Japan** do hereby declare:

1. That I am an inventor of the above-identified application.
2. That I graduated from Tokyo Metropolitan University in March, 1986, with a Masters degree in Science.
3. Beginning in April 1986, I have been engaged in research and development works related to biologically active substances. In April 1993, I began my employment at the Agency of Industrial Science and Technology, which is the predecessor of the National Institute of Industrial Science and Technology (the Assignee). From April 1993 onwards, I have been mainly engaged in research and development works related to the utilizability and applicability of marine algae.
4. I declare that I am one of the authors of Hirotaka et al. (Bulletin of the Society of Sea Water Science, Japan, 2000, 54:310-315) (hereinafter referred to as Hirotaka (2000)) and Hirotaka (Heisei 10-12, Nendo Kagaku Gijutsu Sogo Kenkyu Itakuhi, Chiiki Sendo Kenkyu, Kenkyu Seika Hokokusho, Zaidan Hojin Kochiken Sangyo Shinko Center, 2001, 176-192)

(hereinafter referred to as Hirota (2001)). Such references were relied upon by the Office in rejections of claims 1-3 and 10-12 under 35 U.S.C. § 102(b) or 35 U.S.C. § 103(a) on pages 5-7 of the Office Action dated July 24, 2008.

I note that in items 11 and 12 on pages 6 and 7 of the Office Action dated July 24, 2008, the Office indicates that Hirota (2000) and Hirota (2001) teach a culture strain isolated from the same source and using similar techniques as the claimed culture strain. Thus, it is the Office's position that the strain of Hirota (2000) and Hirota (2001) inherently possess the claimed characteristics. It is my expert opinion and belief that the strain of these references do not possess all the claimed characteristics of the claimed unialgal culture strains. I further declare that the culture strain of these references is the same culture strain as the culture strain used in Comparative Example 1. That is, the culture strain was obtained from a marine alga of the genus of *Gracilaria* sp. growing in the coast adjacent to the estuary of the Yoshino River, which was referred to as *Gracilaria chorda* (growing in the estuary of the Yoshino River). Please see [0037] of the specification.

The following experiments comparing the claimed culture strain to that of the cited references was performed under my direction and control. I further note that such data is also shown on pages 15-17, 23 and 24 of the specification.

EXPERIMENTAL DATA

Growth and Maturation Evaluation of Unialgal Culture Strain

An alga culture test instrument capable of temperature control (temperature distribution: $\pm 0.5^{\circ}\text{C}$), light intensity control (stepless light control), and day length control was used to evaluate the maturation of the unialgal culture strain. This instrument could simultaneously be applied to fifty 500-ml Erlenmeyer flasks to culture (dimension in the tank: 1250 mm wide x 720 mm deep x 900 mm high). Apical fragments of 4 mm in length were prepared from the unialgal culture strain of the marine macroalga *Gracilaria chorda* and added at 6 fragments per Erlenmeyer flask containing 400 ml of culture seawater. Irradiation conditions were set to conditions of a 14-hour light phase and a 10-hour dark phase, and the culture solution was replaced once a week with fresh one. The number of experimental samples under the identical culture condition was 5.

Subsequently, the evaluation of maturation of the unialgal culture strain was performed with aeration under 11 conditions in total of (i) 6 levels of temperature (with an increment of 4 °C from 10 to 30 °C) under the constant light intensity condition of 60 $\mu\text{mol}/\text{m}^2/\text{sec}$ and (ii) 5 levels of light intensity (with an increment of 20 $\mu\text{mol}/\text{m}^2/\text{sec}$ from 20 to 100 $\mu\text{mol}/\text{m}^2/\text{sec}$) under the temperature condition of 22 ± 0.5 °C.

The replacement of the culture solution and the measurement of a marine alga wet mass were performed in a clean booth. In this way, the presence or absence of maturation was determined by recording a marine alga wet mass per flask while observing the presence or absence of formation of reproductive organs such as cystocarp, tetrasporangium, or spermatogonium on the marine alga surface with a microscope.

As a result, an experimental group of matured marine algae was not observed even after 12 weeks of culturing. At the point in time when the marine alga wet mass per 500-ml Erlenmeyer flask reached 0.2 g, the strain was thinned out to 0.02 g to continue the culture. However, the strain was not matured even after 3 years from the initiation of the culture (initiation of the step (5)).

Growth Rate

A relative growth rate (RGR) is expressed as R. When a marine alga wet mass at the start of culture and a marine alga wet mass after t days of culture were defined as W_0 and W_t , respectively, the relative growth rate is determined according to the equation $R = (\ln W_t - \ln W_0)/t$. The growth rate (%/day) was calculated by multiplying R by 100.

The growth rate of the unialgal culture strain of *Gracilaria chorda* (growing in the estuary of the Katsuura River) in the period of two weeks through three weeks of culture was the highest under the condition of the temperature of 22 °C and light intensity of 60 $\mu\text{mol}/\text{m}^2/\text{sec}$ among the experimental groups, and the value thereof was 14.4%/day.

Growth and Maturation Evaluation with 20 liters of Culture Solution

The unialgal culture strain of *Gracilaria chorda* (growing in the estuary of the Katsuura River) was cultured in ten 1-liter flat-bottomed flasks and grown to a wet mass of 4 g or more. Conditions for this culture were set to the conditions that gave the highest growth rate in the culture on the scale of 400 ml of culture solution, that is, "a temperature of 22°C, light intensity

of $60 \mu\text{mol}/\text{m}^2/\text{sec}$, a light cycle of a 14-hour light phase and a 10-hour dark phase, all-day aeration, and the replacement of the culture solution in a frequency of once a week".

Hereinafter, these culture conditions are referred to as the growing culture conditions.

The culture solution (seawater medium) was prepared by filtering seawater collected in waters with a depth of 1.5 m in Yashima Bay, Takamatsu city, Kagawa prefecture, Japan, with a $0.20\text{-}\mu\text{m}$ cellulose acetate membrane filter (manufactured by Advantec Toyo), then supplementing and mixing the filtrate with 1/10 volume of distilled water, and sterilizing the mixture at 100°C for 30 minutes, to which Provasoli's enrichment agent for seawater sterilized in advance was then added. Hereinafter, this culture solution (seawater medium) is referred to as the seawater for growing culture.

The unialgal culture strain (4 g) of *Gracilaria chorda* (growing in the estuary of the Katsuura River) obtained by growing and culturing were inoculated into a 3D-liter culture container with 20 liters of seawater for growing culture and cultured for 4 weeks under the growing culture conditions. After 4 weeks, the marine alga wet mass was increased by approximately 12 times to approximately 47 g.

No experimental group showing matured marine algae could be found even after 12 weeks of culturing. At a moment thereafter when the marine alga wet mass in the 30-liter culture container with 20 liters of seawater for growing culture had reached 300 g, the strain was thinned out to 10 g to continue the culture. Nevertheless, the unialgal culture strain was not matured even after 3 years from the initiation of the culture. The growth rates of the unialgal culture strain in 400 ml of culture solution and in 20 liters of culture solution, marine alga yields, and the presence or absence of maturation are shown in Table 4.

Table 4

	Culture in 400 ml of culture solution		Culture in 20 liters of culture solution	
	Growth rate in the period of two weeks through three weeks; %/day	Presence or absence of maturation	Change in marine alga wet mass (in four weeks culture)	Presence or absence of maturation
Unialgal culture strain (Example 2)	14.4	Not matured after 3 years	Increase from 4 g to 47 g in 4 weeks culture	Not matured after 3 years
Unialgal culture strain (Comparative Example 1)	8.2	matured on the 12th week of culture	Increase from 4 g to 12 g in 4 weeks culture	matured on the 12th week of culture
Unialgal culture strain (Comparative Example 2)	7.7	matured on the 11th week of culture	Increase from 4 g to 11 g in 4 weeks culture	matured on the 11th week of culture

Absorption of Nutritional Salts

Since marine macroalgae have the ability to absorb nutritional salts such as nitrate nitrogen, phosphate ions, ammonium ions (nitrogen), the maximum amount of nitrate nitrogen absorbed per day was evaluated as the ability of the unialgal culture strain to absorb nutritional salts.

The maximum loading of nitrate ions per unit wet mass on the fourth week of culture of the unialgal culture strain prepared from the spores of the marine alga of the genus of *Gracilaria* sp. growing in the Katsuura River (*Gracilaria chorda* growing in the Katsuura River) was approximately 0.4 mg of nitrogen/g of marine alga wet mass/day. The result is shown in Table 8. The maximum daily loading of nitrate ions per unit wet mass on the third year of culture thereof was also approximately 0.4 mg of nitrogen/g of marine alga wet mass/day.

Table 8

		The maximum loading of nitrate nitrogen absorbed per day
		(mg of nitrogen/g of marine alga wet mass/day)
Unialgal culture strain (Example 2)	on the fourth week of culture	0.4
	on the third year of culture	0.4
Unialgal culture strain (Comparative Example 1)	on the fourth week of culture	0.2
Unialgal culture strain (Comparative Example 2)	on the fourth week of culture	0.1

Thus, as shown in Table 4 above, the unialgal culture strain of the cited references (Comparative Example 1) matured in twelve weeks while the claimed unialgal strain did not mature after three years of initiation of the culture.

I also note that Table 8 shows the results of an evaluation test on nutritional-salt absorbing ability. Absorption of such salts indicate growth. Thus, Table 8 taken with the results of Table 4 shows that the claimed strain is still growing without maturity for three years.

I therefore note that the unialgal culture strain of the cited references fail to teach or suggest each and every element of the claimed invention. In particular, the isolated immaturable unialgal culture strains of the claimed invention has the property of not maturing even after three years of storage or continuous culture while the unialgal culture strain of the cited references matured by about 12 weeks.

Finally, I note that in the final Office Action dated July 24, 2008, in item 12, the Office alleges that Hirotaka (2001) teaches continuous cultivation of unialgal culture strains as a stock

unialgal culture strain. However, as an author of this reference, I note that the "continuous cultivation" described in Hirotaka (2001) and relied upon by the Office in making this contention means a so-called "stock culture" which is a standard technique for preparing a unialgal culture strain for use in evaluation steps. I attest that such corresponds to steps 3 and 4 in paragraph [0048] and [0049] of the specification. Such language does not indicate a unialgal culture strain that can be cultured for a long period of time without maturation.

I further declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Date: November 17, 2008

Hirotaka Kakita
(Signature of Declarant)